Applicants
 :
 Lie-Fen Shyur et al.
 Attorney Docket No.: 70002-111001

 Serial No.
 :
 10/773,455
 Client Ref. No.: 14A-890529

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AMENDMENTS TO THE SPECIFICATION:

Please replace the paragraph beginning at page 6, line 5 with the following amended paragraph:

A nucleic acid was amplified from the full-length Fibrobacter succinogenes 1,3-1,4-β-D-glucanase (Fsβ-D-glucanase) cDNA (Chen et al. (2001), J. Biol. Chem. 276, 17895-17901) by the PCR using the following two primers: Oligo A: 5'-CAGCCGGCGATGGCCATGGTTAGC GCA-3' (SEQ ID NO: 17) and Oligo B: 5'-CTGCTAGAAGAATTCGGAGCAGGTTCGTC-3' (SEQ ID NO: 18). The amplified nucleic acid encodes a polypeptide that corresponds to a fragment from aa 24 to 272 of SEQ ID NO: 1, except that the N24 was replaced with M. The polypeptide lacks the Cterminal 78 aa of Fsβ-D-glucanase. To generate an expression vector, the amplified nucleic acid was digested with Nco I and Eco RI and then ligated into a pET26b(+) vector (NOVAGEN, WI) that had been digested with the same enzymes. The resultant vector was confirmed by DNA sequencing. This construct, designated as pPCR-TFglucanase, encodes a fusion protein (SEQ ID NO: 10) that has a pel B leading peptide sequence (KYLLPTAAAGLLLLAAQPAMA, SEQ ID NO: 11) at the N-terminus and a 19-residue segment (SEQ ID NO: 16) at the C-terminus. Once expressed in a host cell, the pel B leading peptide sequence was cleaved to generate a mature fusion truncated glucanase, PCR-TF-glucanase (SEO ID NO: 9).

Please replace the paragraph beginning at page 6, line 25 with the following amended paragraph:

Another truncated Fsβ-D-glucanase (SEQ ID NO: 7), designated as "TF-glucanase," was created using PCR-based site-directed mutagenesis. This TF-glucanase lacks the just-described 19-residue segment at its C-terminus. To make a nucleic acid encoding it, a stop codon was introduced right after the codon for P248 of the just-described pPCR-TF-glucanase. A pair of complementary mutagenic primers were used. The sense strand primer has the sequence: 5°-CCTGCTCCGTAATCGAGCTCC-3°(SEQ ID NO.: 19). The mutagenesis was carried out in a PCR reaction mixture containing 10

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mM KCl, 10 mM (NH₄)₂SO₄, 20 mM Tris-HCl (pH 8.8), 2 mM MgCl₂, 0.1% Triton^R X-100, 0.1 mg/ml nuclease-free BSA, 10-15 ng of template DNA, 0.2 mM dNTPs, 0.25 μM each of the primers, and 2.5 units of Turbo *Pfu* DNA polymerase (STRATAGENE, La Jolla, CA). The PCR reactions were conducted on a Hybaid TouchDown thermal cycler using the following program: 2 min at 95°C, 16 cycles of 1 min at 55°C/13 min at 68°C/45 sec at 95°C. The products were digested with 10 units of Dpn I at 37°C for 1 hour (h) and subsequently transformed into *E. coli* XL-1 Blue competent cells by electroporation. The transformed cells were grown on LB agar plates containing 30 μg/ml kanamycin at 37°C until colonies appeared on the plates. The colonies were selected randomly and cultured in 5 ml LB/ kanamycin liquid culture at 37°C for 16 h before plasmids were isolated from the culture using a QIAPREP Spin Miniprep kit (QIAGENE, Hilden, Germany). Mutation was confirmed by DNA sequencing. The plasmid thus obtained was named "pTF-glucanase."

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